



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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In re the Application of

Andrew Dames et al.

Serial No. 09/787,195

Filed: September 17, 1999

For: Bio-Assay Technique

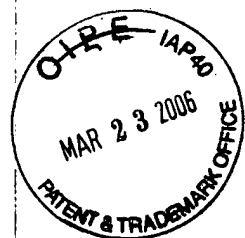
Examiner: Lyle A. Alexander

Group Art Unit: 1743

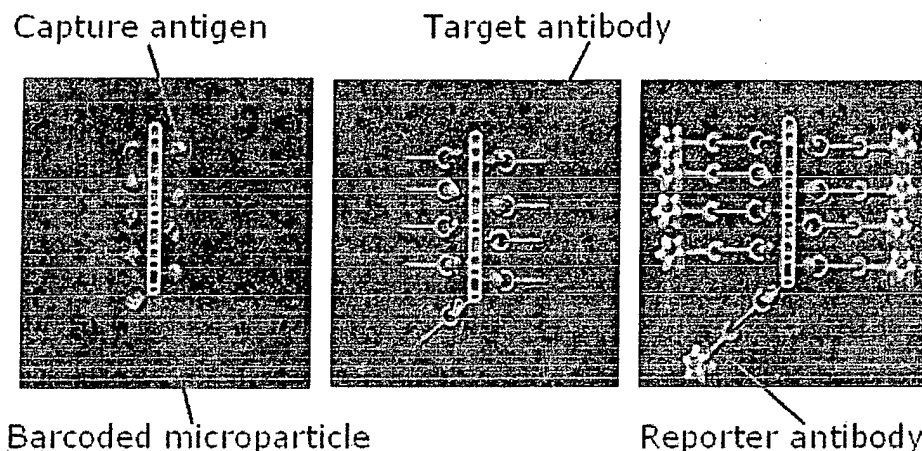
DECLARATION

I, Peter Swarbrick, hereby declare that:

1. I am a citizen of The United Kingdom and reside at 3 College Rd, Impington, Cambridge, UK, CB4 9PL.
2. I received a Ph.D. degree in 1995 from the University of Otago, Dunedin, New Zealand, on the molecular characterisation of the MHC class II DRB genes of red deer, allele association studies for susceptibility to TB, and phylogenetic analysis of the alleles. From 1995 to 1996 I worked as a research scientist for Genomnz, Mosgiel, New Zealand and was responsible for the development of a microsatellite based, commercial, parentage test for deer. From 1997 to 2002 I worked for Incyte Genomics (formerly Hexagen), Cambridge, UK as Project Manager for Incytes' commercial, candidate gene, single nucleotide polymorphism (SNP) discovery programs and as Associate Director with responsibility for Incyte's SNP discovery process. From 2002 to the present I have held the post of Director of Research and Development at SmartBead Technologies Ltd, Cambridge, UK. I have published over 20 research papers.
3. I have read and am familiar with US patent application no. 09/787,195 entitled "Bio-Assay Technique" (hereinafter the '195 application). I have also reviewed the Office Action dated August 23, 2005 in the '195 application, in which the Examiner contends that the claimed solid support is obvious over EP 0395300, US 5129974 (Aurenus) or Rigby et al., "An Anodizing Process for the Production of Inorganic Microfiltration Membranes", Trans. Inst. Metal Finishing, 68 (1990) August, Part 3, London, GB. As a scientist having knowledge, skill and experience in the field of the invention of the '195 application, I do not concur with the Examiner's contention. In particular, considerable advantages are associated with the claimed support, especially because of the limitation to "a largest external dimension ... of less than 100  $\mu$ m". These advantages, in my view, are indicative that the claimed support is not an instance of mere miniaturisation of prior art devices.
4. The claimed support is for performing biochemical assays. In particular, the support, by incorporating a spatially varying identification pattern, enables multiplexed assays. Typically, in such assays, a plurality of the supports are exposed to the same analyte solution, e.g. a patient sample, but different portions of the plurality of the supports have different surface treatments so that only those supports whose surface treatment chemically or biochemically reacts with actual analyte(s) in the solution will bind to that analyte(s). The identification pattern then comes into play because, by recognising supports with a bound analyte and reading the identification pattern on the supports thus-recognised, a user can determine which analyte(s) is actually in the solution.



5. The main steps in such an assay, where for example the support is a barcoded microparticle and the analyte is a target antibody, are shown schematically in the following figures. Firstly capture antigens are bound to the microparticles, different antigens being associated with different barcode numbers. The microparticles representing the various barcode/antigen pairs are mixed in a reaction vessel with a sample which may contain the target antibody. If present, the target antibody binds to its associated capture antigen. A reporter antibody which has a fluorescent dye tag and which binds to antibodies is then added to the reaction vessel. Finally, a reader system identifies all the microparticles present, reads each of their barcodes and measures the amount of reporter antibody, via fluorescence, associated with each barcode. This determines the respective antigen and hence the antibody in the sample.



6. Now, typically, only a small amount of experimental sample or, in the case of clinical diagnostics, patient sample, is available and so it is essential that efficient use is made of it. Also, in general, it is desirable to perform as few assays as possible in order to reduce costs, save time and, in the case of clinical diagnostics, provide early patient diagnosis. So the power of such an approach depends critically on the ability to multiplex the assay. That is, it depends on having in each reaction vessel sufficient identification pattern/surface treatment pairs (i.e. barcode/antigen pairs in the above example) to address the number of possible analytes (target antibodies in the above example) in the sample. Furthermore, for a statistically robust assay, in general at least 10 supports of each identification pattern, and preferably at least 20 supports, should be readable by the reader system. Also, in practise, the wells of industry standard 96-well plates are used as standard reaction and reading vessels. Such wells are normally cylindrical in shape with transparent bottoms, and a usual way to read the supports is optically through the well bottoms, but the surface area of each well bottom is typically only about 30 mm<sup>2</sup>.
7. It follows from the above that support size is a crucial factor. Aurenus' microlabels are the smallest of the articles disclosed by EP 0395300, Aurenus and Rigby et al. but are still about 1 mm × 1 mm (i.e. 1 mm<sup>2</sup>) in overall size (see Aurenus, column 4, lines 46-48). If we assume that these microlabels are optimally arranged side-by-side with no overlapping on such a well bottom, it is evident that a maximum of only about 30 microlabels could be viewed through the well bottom. In contrast, a linear support according to present claim 1 and as described in the detailed description and illustrated in Figure 1 of the '195 application may have a length of about 100 μm, width of 10 μm



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and thus an area of about  $0.001 \text{ mm}^2$ . Thus if they are optimally arranged, of the order of about 30,000 such supports could be viewed through a well bottom. Of course, in practise, some overlapping of supports inevitably occurs, but nevertheless the calculations illustrate that the claimed support has the ability to offer something like a thousand-fold increase in the number of viewable supports compared to Aurenus' microlabels.

8. Thus, were Aurenus microlabels to be used in a multiplexed assay in a well of a standard 96-well plate (and I do not admit that it would be obvious or even feasible to do this), at best only a three-plex assay could be performed. In contrast, adopting a support of the present invention would allow a 3,000-plex assay to be performed, or, for more robust statistics, a 300-plex assay could be performed with about 100 supports of each identification pattern available for reading by the reader system. Further substantial improvements in multiplexing capability would be obtainable by adopting, say, a  $70 \mu\text{m} \times 8 \mu\text{m}$  support, as currently under development by the assignee of the '195 application, SmartBead Technologies Ltd. This support has an area of  $0.00056 \text{ mm}^2$ , allowing about 50,000 supports to be viewed through a well bottom.
9. Of course, even outside the multiplexing issue, it is evident that a hypothetical assay based on Aurenus microlabels, because of their greater surface area and volume, would require much greater volumes of sample and reagent than would be required by an assay based on the claimed supports. Also it is doubtful that Aurenus microlabels, because of their size, could flow through the channels of standard flow-cytometers were it desired to use such equipment in the performance of an assay.
10. Overall, and particularly when multiplexing is considered, the claimed support, because of its size, provides significant advantages which are not obtainable by the device/microlabel/membrane of EP 0395300, Aurenus and Rigby et al., or even hinted at by their respective disclosures.
11. I further declare that all statements made herein of my knowledge are true, and that all statements made on information and belief, including those that can be supported by citations to published scientific literature, are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the '195 application or any patent issued thereon.

22-12-05  
DATE

  
PETER SWARBRICK, Ph.D.